UNIVERSITI PUTRA MALAYSIA

# ENZYMATIC EXTRACTION AND MODIFICATION, AND FRYING STABILITY OF MORINGA OLEIFERA SEED OIL

ABDULKARIM SABO MOHAMMED.



# ENZYMATIC EXTRACTION AND MODIFICATION, AND FRYING STABILITY OF *Moringa oleifera* SEED OIL

By

ABDULKARIM SABO MOHAMMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2006

**DEDICATIONS**

*This piece of work is dedicated to my parents, wife and children.*

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

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**January 2006**

**Chairman:** **Professor Hasanah Mohd. Ghazali, PhD**

**Faculty:** **Food Science and Technology**

Consumption of edible oils has grown with the increase in world population. The increasing health awareness and consciousness amongst consumers made the food industry more discriminating in the types of oil they use for food applications. Many circumstances have focused attention on high-oleic vegetable oils, which have been demonstrated to reduce the risk of coronary heart disease. The demand for high-oleic oils is increasing but there are only a few known sources available. *Moringa oleifera* seed oil, which is naturally high-oleic oil, therefore, presents a great opportunity for the oil industry for meeting this ever-increasing demand.

The objectives of this study were to determine the properties of oil extracted from *Moringa oleifera* seeds, evaluate the efficiency of enzymatic-extraction of the oil and modification of the oil to enhance its oleic acid content and compare the oxidative stability of the oil against several other oils during deep fat frying.

The oil content of *M. oleifera* seeds in Malaysia ranged between 30.8% and 33.4% depending on the variety, of which there were two. The physico-chemical properties of the oil were determined following extraction with light petroleum ether. The dominant fatty acid (FA) of the oil was indeed oleic acid, where Variety 1 contained 67.9% while Variety 2 contained 74.4%. After refining, the oil from both varieties is light golden color (0.1R + 1.0Y), and a viscosity, smoke point and refractive index ($n_D$40°C) of Cp 51.7, 206°C, and 1.4533, respectively. Using electronic nose analysis, the crude oil was found to have an odor similar to that of peanut oil. It has a complete melting point of 18.9°C. The crude oil contains 95.6% triacylglycerols (TAG) and 1.9% 1,2- and 1,3-diacylglycerols. The relative TAG content increased to 98.7% after refining. The oil contains 36.7% trioleoyl glycerol (OOO) as the main TAG.

*M. oleifera* seed oil was extracted using four different types of enzymes namely; Neutrase 0.8L (neutral protease), Termamyl 120L, type L (α-amylase), Pectinex Ultra SP-L (pectinase) and Celluclast 1.5L FG (cellulase) all supplied by Novozymes (Bagsvaerd Denmark). The enzymes were used either separately or in combination. The efficiency of enzyme-extraction was compared to aqueous extraction without enzyme. Enzymatic-extraction of *M. oleifera* seed oil showed that Neutrase alone at 2% v/w, 45°C and pH 6.8 was able to extract 71.9% oil relative to the amount obtained when the oil was solvent-extracted. Neutrase was the most efficient among the enzymes used followed by Termamyl, Celluclast and Pectinex with percent oil recoveries of 64.8%, 62.6% and 56.5%, respectively. Each extraction was carried out at the optimum pH and temperature of the enzymes. A combination of the four enzymes at pH 7.5 increased the

oil recovery to 74%. Percent oil recovery with all enzymes was significantly ($P<0.05$) higher than the control (aqueous extraction without enzyme) (35.6%).

Solvent extracted *M. oleifera* seed oil was transesterified using immobilized lipase (Lipozyme IM 60) (Novozymes Bagsvaerd Denmark) in order to change its melting and crystallizing behavior that will make it easier to fractionate. After transesterification, the oil was fractionated with acetone at -18°C and without acetone at 10°C to obtain two fractions, stearin and olein fractions. Incubation of the transesterified oil at 10°C for 24 h resulted in the formation of fat crystals, which settled at the bottom of the flask in sample transesterified for 24 h, while the control (0 h) sample became rather viscous with fat crystals in suspension. Transesterification affect the TAG profile of the oil, which in turn affected the solid fat content (SFC) and thermal behavior. The SFC value at 0°C after 24 h of reaction was 10.35% and significantly ($P<0.05$) higher than the control (0 h) (7.94%). The oil remained liquid at 20°C for all reaction times. The end set temperature (melting point) shifted from 18.9°C for the unreacted oil to 20.5°C for oil transesterified for 24 h. Transesterification of the oil resulted also in a significant ($P<0.05$) increase in the crystallization temperature of the high melting glyceride from the original value of 1.6°C to 12.9°C after transesterification for 24 h. There was a significant increase in the oleic acid content in the olein fractions obtained following fractionation of the transesterified oil with and without using acetone (75.2 and 70.5%, respectively) compared to the unreacted oil (67.9%).

The oxidative stability of refined *M. oleifera* seed oil (MoO) in deep fat frying was evaluated and compared with canola (CLO), soybean (SBO), and palm olein (PO). The oils were used to fry potato chips for 6 h a day up to a maximum of 5 days. Changes in fatty acid (FA) composition, free fatty acids (FFA), iodine value (IV) peroxide value (PV), *p*-anisidine value (*p*-AV), specific extinction ($E^{1\%}_{1cm}$ 233 and 269 nm for conjugated dienes and trienes), total polar compounds (TPC), color and viscosities were used to evaluate the oils.

The frying process caused an increase in the FFA contents MoO, PO, CLO and SBO. The FFA contents at the end of the frying period were 0.35%, 0.55%, 0.54% and 0.51% for CLO, PO, SBO and MoO, respectively. The rate of increase in the PV ($meqO_2/kg$) for CLO (2.33 per day) was higher compared to those of MoO (0.80 per day), PO (1.00 per day), and SBO (0.70 per day). Conjugated dienes levels at the end of the frying period were lowest in PO (4.27) followed by MoO (6.07) with high levels in CLO (9.28) and SBO (10.64). The amount of TPC in MoO (20.78%) and PO (21.23%) were significantly ($P<0.05$) lower than those in CLO (28.73%) and SBO (31.82%). Color and viscosity of the oils increased with frying time. The rates of change of viscosity with the frying days were similar for all the oils. Results of sensory analysis conducted on potato chips fried in PO and MoO showed general acceptability of potato chips fried in both oils with high scores for crispness (7.07 and 7.14), oiliness (6.86 and 7.09), and fried food flavor (7.00 and 6.79) attributes, respectively. The overall acceptance of the French fries fried in MoO was high (7.50) and not significantly ($P>0.05$) from that of PO (7.58).

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah.

**PENGEKSTRAKAN BERENZIM, PENGUBAHSUAIAN BERENZIM DAN KESTABILAN PENGGORENGAN MINYAK BIJI *Moringa oleifera.***

Oleh

**ABDULKARIM SABO MOHAMMED**

**Januari 2006**

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Penggunaan minyak masak telah berkembang seiring dengan peningkatan bilangan penduduk dunia. Peningkatan tahap kesedaran terhadap kesihatan di kalangan pengguna menjadikan industri makanan semakin memilih tentang jenis minyak yang digunakan untuk aplikasi makanan. Banyak keadaan telah menumpukan perhatian kepada minyak sayuran tinggi asid oleik di mana minyak begini telah dibuktikan dapat mengurangkan risiko penyakit koronari jantung. Permintaan minyak tinggi asid oleik yang semakin meningkat tetapi sumbernya adalah terhad. Secara semulajadinya, minyak biji *M. oleifera* adalah minyak tinggi asid oleik, dan ini memberi peluang yang cerah kepada industri minyak untuk memenuhi permintaan ini.

Objektif kajian yang dijalankan ini adalah untuk menentukan ciri-ciri minyak yang diekstrak dari biji *Moringa oleifera*, menilai keberkesanan pengekstrakan minyak secara berenzim dan pengubahsuaian minyak tersebut secara berenzim untuk meningkatkan

kandungan asid oleik, dan membandingkan kestabilan pengoksidaan minyak tersebut dengan beberapa minyak lain semasa penggorengan dalam minyak penuh.

Minyak biji *M. oleifera* diekstrak menggunakan empat jenis enzim yang berbeza. Pengekstrakan berenzim telah dibandingkan dengan pengekstrakan tanpa enzim melalui menggunakan empat jenis enzim iaitu Neutrase 0.8L (protease neutral), Termamyl 120L, type L (α-amylase), Pectinex Ultra SP-L (pectinase) dan Celluclast 1.5L FG (cellulase) yang dibekalkan oleh Novozymes (Bagsvaerd Denmark). Enzim ini digunakan sama ada secara tunggal atau digabungkan. Kandungan minyak biji *M. oleifera* di Malaysia didapati dalam julat 30.8% dan 33.4%, bergantung kepada jenis varieti, di mana terdapat dua jenis varieti. Ciri-ciri fisiko-kimia minyak ditentukan setelah pengekstrakan menggunakan petroleum eter. Asid lemak paling utama adalah asid oleik, di mana Varieti 1 mempunyai sebanyak 67.9%, sementara Varieti 2 mempunyai sebanyak 74.4% asid oleik. Selepas penyulingan, minyak dari kedua-dua jenis varieti ini mempunyai warna cerah keemasan (0.1R + 1.0Y) dan nilai kelikatan, takat wasap dan indeks pembiasan iaitu ($n_D$40°C), Cp 51.7, 206°C dan 1.4533, masing-masing. Dengan menggunakan analisis hidung elektronik, minyak mentah didapati mempunyai bau yang seakan sama dengan minyak kacang. Takat leburnya ialah 18.9°C. Minyak kasar tersebut mengandungi 95.6% triasilgliserol (TAG), 1.9% 1,2- dan 1,3-diasilgliserol. Kandungan relatif TAG meningkat kepada 98.7% selepas penyulingan. Minyak tersebut mengandungi 36.7% triolioylgliserol (OOO) sebagai TAG yang utama.

Pengekstrakan berenzim minyak biji *M. oleifera* menunjukkan bahawa Neutrase (protease neutral daripada Novozymes Bagsvaerd Denmark) adalah paling berkesan dengan 71.9% penghasilan minyak, diikuti dengan Termamyl (α-amylase) (64.8%) Celluclast (cellulase) (62.6%) dan Pectinex (pectinase) (56.5%) dengan setiap pengekstrakan dijalankan pada pH dan suhu yang optimum bagi ke semua enzim tersebut. Gabungan empat jenis enzim berkenaan adalah lebih berkesan daripada penggunaan enzim secara bersendirian dengan penghasilan sebanyak 74%. pH yang digunakan adalah optimum bagi Neutrase. Peratus penghasilan minyak dengan kesemua enzim tersebut signifikannya ($P<0.05$) lebih tinggi berbanding kawalan (pengekstrakan akues tanpa enzim) (35.6%).

Minyak biji *M. oleifera* yang diekstrak dengan pelarut telah ditransesterifikasi menggunakan lipase tersekat-gerak (Lipozyme IM 60) dengan tujuan menukar pelakuan penghabluran yang memudahkannya untuk dipisahkan. Selepas ditransesterifikasi, minyak tersebut dipisahkan dengan aseton pada suhu –18°C dan tanpa aseton pada suhu 10°C untuk memperolehi dua pecahan; pecahan stearik dan olein. Pengeraman minyak teresterifikasi pada 10°C selama 24 jam menyebabkan pembentukan hablur-hablur lemak yang termendak di dasar kelalang sementara minyak kawalan (0 jam) pula menjadi agak likat dengan hablur-hablur lemak yang terampai. Transesterifikasi menjejaskan profil TAG minyak tersebut di mana ia turut menjejaskan nilai kandungan lemak pepejal (KCP) dan pelakuan haba. Nilai KCP pada 0°C selepas tindakbalas 24 jam adalah 10.35% dan adalah lebih tinggi ($P<0.05$) daripada kawalan (0 jam) (7.94%). Minyak tersebut kekal cair pada 20°C pada keseluruhan masa tindakbalas. Suhu akhir

yang ditetapkan (takat lebur) meningkat daripada 18.9°C bagi minyak tanpa tindakbalas kepada 20.5°C bagi minyak yang telah diesterifikasikasi selama 24 jam. Transesterifikasi minyak juga mengakibatkan peningkatan yang bererti ($P<0.05$) ke atas suhu penghabluran bagi takat lebur tinggi gliserida daripada nilai asalnya iaitu 1.6°C kepada 12.9°C selepas transesterifikasi selama 24 jam. Berlaku pertambahan yang bererti ke atas kandungan asid oleik dalam pecahan olein yang diperolehi berikutan dengan pemisahan minyak yang diesterifikasi dengan dan tanpa aseton (75.2% dan 70.5%) berbanding dengan minyak asal yang tidak ditindakbalas.

Kestabilan pengoksidaan minyak biji *M. oleifera* yang telah ditulenkan dalam penggorengan minyak penuh telah dinilai dan dibandingkan dengan minyak kanola (CLO), minyak kacang soya (SBO) dan minyak kelapa sawit (PO). Minyak-minyak tersebut digunakan untuk menggoreng kentang selama 6 jam sehari sehingga maksimum 5 hari. Perubahan komposisi asid lemak, asid lemak bebas, nilai iodin, peroksida (PV), *p*-anisidin (*p*-AV), nilai pelupusan spesifik ($E^{1\%}_{1\,cm}$ 233 dan 269 nm), jumlah sebatian polar (TPC), warna dan kelikatan telah digunakan untuk menilai minyak tersebut.

Aktiviti pengeringan yang dijalankan telah menyebabkan peningkatan kandungan FFA bagi minyak MoO, PO, CLO dan SBO. Kandungan FFA pada akhir masa penggorengan masing-masing adalah 0.35%, 0.55%, 0.54%, dan 0.51% bagi CLO, PO, SBO, dan MoO. Kadar pertambahan dalam nilai PV ($meqO_2/kg$) bagi CLO (2.33 per hari) adalah lebih tinggi berbanding dengan minyak MoO (0.80 per hari), PO (1.00 per hari) dan SBO (0.70 per hari). Paras diene konjugat pada akhir masa penggorengan adalah paling

rendah dalam PO (4.27) diikuti dengan aras tertinggi bagi CLO (9.28) dan SBO (10.64). Jumlah TPC dalam MoO (20.78%) dan PO (21.23%) adalah lebih rendah secara bererti ($P<0.05$) berbanding dengan CLO (28.78%) dan SBO (31.82%). Warna dan kelikatan bagi minyak tersebut juga meningkat dengan masa penggorengan. Kadar perubahan kelikatan dengan masa penggorengan adalah hampir sama dengan kadar ke semua minyak tersebut. Keputusan analisis sensori menunjukkan penerimaan keseluruhan terhadap kentang yang digoreng dalam MoO dan PO, masing-masing, memberi markah tinggi bagi ciri-ciri seperti kerangupan (7.07 dan 7.14), rasa minyak (6.86 dan 7.09), dan citarasa makanan bergoreng (7.00 dan 6.79). Penerimaan keseluruhan terhadap kentang goreng dalam MoO adalah tinggi (7.50) dan tidak bererti ($P>0.05$) dengan PO (7.58).

## ACKNOWLEDGEMENT

In the name of Allah most beneficent most merciful. All praise be to Allah for all the favors bestowed upon Mankind. I wish to start by expressing my sincere gratitude to my supervisor, Professor Hasanah Mohd Ghazali of the Department of Food Science, Faculty of Food Science and Technology for all the support and guidance she offered throughout the period of this study. She made it possible for me to fulfill my ambition of studying for this degree by providing all the support and for been there for me all the time throughout the study period. I am indeed very grateful to my research committee members, in the names of Associate Professors Dr. Lai Oi Ming of Bioprocess Technology Department, Faculty of Biotechnology and Biomolecular Sciences, Sharifah Kharidah Syed Muhammad of Food Science Department, Faculty of Food Science and Technology and Dr. Kamariah Long of Malaysian Agricultural Research and Development Institute for their untiring support and advises which made this research feasible.

I would like to thank the technical and administrative staff of the Faculty of Food Science and Technology and the research staff of MARDI for their assistance. I am very thankful to my fellow graduate students in enzyme technology laboratory for being very friendly and supportive during difficult times.

I wish to acknowledge the Malaysian government for the IRPA grant awarded to Professor Dr. Hasanah Mohd Ghazali, with which this research was conducted and University Putra Malaysia for giving me the opportunity to study for the PhD degree.

Lastly I would like to thank my parents, my wife Fatima and my little Ruqayya for their support and care at all times.

I certify that an examination committee met on 20th January 2006 to conduct the final examination of Abdulkarim Sabo Mohammed on his Doctor of Philosophy thesis entitled "Enzymatic extraction and modification, and frying stability of *Moringa oleifera* seed oil" in accordance with Universiti Pertanian Malaysia (higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommended that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

**ABDULKARIM SABO MOHAMMED**

Date: 10th February 2006

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